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THE WALTER AND ELIZA HALL INSTITUTE OF RESEARCH IN PATHOLOGY AND MEDICINE

THE DIRECTOR'S TWENTIETH ANNUAL REPORT

1938-39

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Historical Preface

The Walter and Eliza Hall Institute of Research in Pathology and Medicine was founded in 1916. The Trustees of the late Walter and Eliza Hall completed the Pathological Block of the present Royal Melbourne Hospital, and provided an annual payment of £2,500 towards the upkeep of the Institute. In 1925 this was increased to £3,100, and since 1930 has been £3,200 annually. In addition, the Institute has received, since its inception, £500 per annum from the University, the Medical Staff of the Melbourne Hospital having agreed that this portion of its clinical fund should be so used. Since 1925 a further sum of £250 per annum has been provided by the University from interest on the Appeal Fund (1924), together with an annual contribution from the Clinical Research Fund.

In 1926 the late Sir Aaron Danks, then President of the Melbourne Hospital, established an Endowment Fund to support a Biochemical Department. This fund has now reached the total of £8,591. The Edward Wilson Trustees maintained this new department by contributing £1,500 per annum in 1926, 1927 and 1928, and £1,000 in 1929. This Trust also, in 1926, contributed £3,000 for the foundation of an Institute Library, and of this sum £2,000 has been invested as endowment.

The Institute has been supported by annual contributions from the Felton Bequest Committee (since 1928), the Trustees of the late Anthony Mackie (since 1933), and the Trustees of the late T. J. Sumner (since 1929). The Trustees of the late Alfred Edments have also helped us since 1937.

The Institute has received substantial support from the Department of Health of the Commonwealth Government; in the years 1927, 1928, 1929, 1930 and 1931 a total of £10,650 was received for special researches. In 1934 the Commonwealth Government entered into an agreement with the Rockefeller Foundation that each would contribute to the Institute the sum of £1,000 per annum for the ensuing three years to support work on Virus diseases, particularly those affecting the central nervous system. In 1937 the Rockefeller Foundation and the Department of Health of the Commonwealth Government agreed to continue their support of this work for a further two years. The National Health and Medical Research Council have provided additional funds during 1938 and 1939, and Mr. E. Alex. Cato has generously arranged to contribute £1000 per annum for the five years' period from 1st January, 1939, to 31st December, 1944.

In 1932, by the will of the late Mrs. L. E. W. Carty, of Brisbane Hill, Hamilton, the Elsie Marion Carty Fund was founded. Mrs. Carty willed to the Institute a portion of her estate and a residuary interest therein to establish this Fund. It was her wish that the income should be used, primarily, for the assistance of promising research workers.

From 1934 to 1937 Mr. A. M. Nicholas gave an annual sum of £250 towards the salary of the Assistant Director, and Mrs. Nicholas generously continued this contribution in 1938 and 1939.

In 1936 the Carnegie Corporation of New York paid the travelling expenses of Dr. W. Feldberg and his family to Australia, and contributed in addition 4,500 dollars, which, with £1,100 collected by Mr. Reuben Hallenstein, made it possible to maintain this worker for two years.

The establishment of the National Health and Medical Research Council in 1937 has aided greatly in the development of this and other institutes for medical research in Australia.

The Institute is controlled by a Board consisting of representatives of the Walter and Eliza Hall Trust, of the University of Melbourne, and of the Committee of Management and Medical Staff of the Royal Melbourne Hospital. During the war years Professor Sir Harry Allen acted as Honorary Director. In 1919 Dr. S. W. Patterson was appointed first Director; in April, 1923, he resigned, and was succeeded by Dr. C. H. Kellaway in August, 1923. During the interval Mr. H. Dew acted as Director.

The Director's Twentieth Annual Report

TO THE BOARD

OF THE

Walter and Eliza Hall Institute of Research in Pathology and Medicine

JULY, 1939.

This year is the last of the period of five years during which the work of the virus department has been financed by annual grants of £1000 from the Rockefeller Foundation and £1000 from the Health Department of the Commonwealth Government. Mr. Alex. E. Cato has generously contributed £1000 per annum for the next five years, and, with the continued support of the National Health and Medical Research Council, it will be possible to continue this important work for a further term.

Dr. Burnet's work on virus diseases received further recognition last year. He was awarded the Burfitt prize and medal of the Royal Society of New South Wales for distinguished work done in Australia during the last three years, and for the researches on Q. fever he shared with Dr. Derrick the Cilento Medal, awarded by the Commonwealth Department of Health for work on tropical hygiene or native welfare.

Dr. Wilhelm Feldberg, who, through the generosity of the Carnegie Corporation of New York and of some local benefactors, had been brought out to Australia, and who had worked in the department of physiology for two years, left us in July, 1938, and has accepted the post of lecturer in Mammalian Physiology at Cambridge. We have not wholly lost contact with him, because he is still engaged on problems similar to those upon which he worked while here, and he is at present carrying out a joint study with Dr. Trethewie and myself upon tissue injury in anaphylaxis.

The work of the Institute has been greatly helped by the strong support of the National Health and Medical Research Council, and much of the work reported here would not have been possible without this help.

DEPARTMENT OF VIRUS RESEARCH.

The year's work of the Virus Department has beeen concentrated largely on two fields, poliomyelitis virus and the group of potentially neurotropic viruses of which that of herpes simplex is the best known. The poliomyelitis work was carried out under a separate grant from the National Health and Medical Research Council, which enabled special animal accommodation to be erected in the grounds of the Veterinary Research Institute at Parkville, and provided an adequate supply of monkeys. In addition to these two main topics, a certain amount of work has been continued in regard to other viruses, mostly in direct continuation of work mentioned in the last Report.

Poliomyelitis.

The serious 1937-38 epidemic of poliomyelitis in Victoria, Tasmania, and South Australia stimulated public interest in the disease, and the funds provided by the National Health and Medical Research Council allowed a fairly extensive programme of laboratory work to be undertaken. The experimental work was carried out by Dr. Burnet and Dr. Jackson, and Dr. Graeme Robertson was responsible for the associated histological investigations. During the course of the epidemic several strains of virus were transferred to monkeys and material stored for subsequent use. With the co-operation of Dr. S. W. Williams, a series of serum samples from patients and contacts were obtained and ampouled. The original objects of the experimental work were first to define the conditions under which serum neutralization tests could be most reliably carried out, and second to determine whether or not the passage of a widespread epidemic over the community resulted in the appearance of antibody in those exposed to infection.

In view of the irregularity of neutralization tests carried out by the standard technique of intracerebral inoculation, an attempt was made to find a better method. In several respects the intraocular route, by which the serum-virus mixture is deposited against the retina, has theoretical advantages, and the possibilities of this method were experimentally studied. The minimal infecting dose by the intraocular route is a little larger than the intracerebral minimal infecting dose, but satisfactory virus emulsions will infect with 0.1 c.c. of a 1:100 dilution. Comparative tests of the neutralizing power of two human serum pools showed that the apparent titre by the intraocular method was higher than by the intracerebral, and the results more consistent. An almost constant initial symptom following infection by this route is a bilateral ptosis, which, with the M.V. strain, usually precedes complete prostration by three or four days. Histologically severe lesions are to be seen in the region of the oculo-motor nuclei.

The intraocular method has been adopted as standard for our investigation of possible changes in antibody content in the sera of children who had been clinically infected or had been in close contact with patients. Two samples of sera from each of twenty poliomyelitis cases and thirteen contacts were available, one taken at the acute stage of illness or soon after contact, the other from six to fifteen weeks later. Our results were fully confirmatory of those obtained by Brodie and collaborators (1937) in showing (a) that several children in the acute stage of poliomyelitis possessed antibody, and (b) that those who possessed none at that stage did not develop any within two to three months after infection. Nor was there any evidence of change in antibody content of the children exposed to contact. It may be noted that one sample of serum was obtained from a contact who subsequently developed poliomyelitis. Both samples gave partial neutralization of about the same degree. In discussing these results it is suggested (1) that in poliomyelitis, a strictly neurotropic infection, very little antigen reaches the antibody-producing mechanism, hence little or no antibody response is likely, (2) as a corollary it is probable that ''poliomyelitis antibody'' has no specific relation to the virus, but is provoked by some unknown micro-organismal antigen which has common antigenic determinant groups.

The Susceptibility of Cynomologus Monkeys to Poliomyelitis.—Owing to a close season for the export of rhesus monkeys from India, a consignment of cynomolgus monkeys was obtained from Singapore. These proved extremely susceptible to the local strain of poliomyelitis virus. In rhesus monkeys this strain caused fairly regular signs of infection, but the degree of paralysis was extremely variable, and eventual recovery was almost the rule. In cynomolgus, all infections appear to be fatal. Both with the local strain and with the strain M.V., much larger amounts of virus are demonstrable in the central nervous system of the paralysed cynomolgus monkeys than in rhesus.

The ease with which infection spreads to the central nervous system from the periphery is noteworthy. In at least seven instances simple swabbing of virus over the pharyngeal region has resulted in infection. In five of these the initial symptoms pointed to a bulbar involvement, suggesting that the virus had passed directly from the pharyngeal region *via* one of the lower cranial nerves to the medulla. Intradermal inoculation also gives a high proportion of infections of the central nervous system. Experiments by Dr. Jackson suggest that the local strain is much more effective in passing to the central nervous system from an intradermal inoculation than the monkey passage strain M.V. Neutralization tests may be carried out as satisfactorily as in the rhesus, and except for a certain tendency for nonspecific deaths to occur after inoculation, this species seems to be preferable to the rhesus as a standard experimental animal for poliomyelitis.

Heterologous Antibodies in Poliomyelitis Sera.—In view of previous work which indicated some relation between susceptibility to poliomyelitis and lack of antitoxic immunity to diphtheria, a series of seventy sera from poliomyelitis patients were tested for their content of four "normal" antibodies, diphtheria, and staphylococcal antitoxins and antibody against the viruses of herpes simplex and swine influenza. It was found that when differences of age and social environment were allowed for, the series showed no significant difference in the proportion with each antibody from that obtained with a control group. The most interesting finding from this work was that antibody against herpes virus and diphtheria antitoxin were much commoner in children from industrial suburbs than in those from residential areas, but that no such relation was evident in regard to influenza virus antibody or staphylococcal antitoxin. On the whole, an examination of the series indicated that each antibody was independently acquired as a result of essentially fortuitous contacts with the corresponding micro-organism, not by any form of maturation. There is a significant but not absolute correlation between poliomyelitis antibody and diphtheria antitoxin, particularly in younger children. This suggests that whatever the agent responsible for the production of poliomyelitis antibody may be, it spreads through the community in very much the same fashion as toxigenic diphtheria bacilli do.

The Herpes Simplex Group of Viruses.

There are three viruses, herpes simplex, B. virus of Sabin and Wright, and pseudorabies virus, which were known to be of generally similar character, and to show rather distant serological relationships. A comparative study of these three viruses has been made by Dr. Burnet, Dr. Jackson, and Miss Lush. All three can be readily grown on the chorioallantois, producing focal lesions of generally similar character. With repeated passage on the chorioallantois, the virulence tends to rise for the chick embryo and to show a slight decrease for other susceptible hosts. The three virus strains used were initially all highly pathogenic for the rabbit, and remained lethal by peripheral inoculation after from twenty to fifty egg passages. The only sharp difference noted was the absence of "mad itch" symptoms in rabbits inoculated with egg passage pseudorabies virus.

Herpes virus is an unusually satisfactory one for pock counting titrations, and a study of the kinetics of the virus-antibody reaction was therefore made along the same lines as were described in Burnet, Keogh, and Lush's (1937) monograph. The results were in accord with other virus neutralization reactions in showing the existence of an equilibrium level reached immediately with a strong serum, but at a detectable rate with more dilute serum.

The Epidemiology of Human Herpes Infections.—The chorioallantoic technique made it possible to carry out a large number of quantitative titrations of the antibody content of human sera. These titrations provided several facts of interest:

(1) With very few exceptions, human sera contained either no herpes antibody at all or an amount sufficient to inactivate more than 99.9 per cent. of a stock virus.

(2) Amongst adult public hospital patients about 93 per cent. of individuals show herpes antibody. Approximately the same proportion obtains amongst children from the industrial suburbs of Melbourne, but infants (under two years) in foundling homes show only a few with antibody.

(3) A survey of sera from a few families in the industrial area showed that, with one exception, only the children under two years had no herpes antibody. By contrast, diphtheria antitoxin usually appeared in early school age.

(4) Adults and children from upper and middle class environments show a distinctly lower proportion with herpes antibody, about 35 per cent.

Following up the suggestion by Dodd, Johnson, and Buddingh that aphthous stomatitis of children is herpetic in origin, a clinical study of this condition was made by Dr. S. W. Williams at the Children's Hospital, the laboratory investigations being carried out at the Hall Institute. It proved easy to confirm the observation that herpes virus could almost invariably be detected in the mouth lesions of stomatitis in infants and very young children. It was found, too, that blood taken at the acute stage contained no herpes antibody, but that it was present in a second sample taken some weeks later.

On the basis of these findings it was concluded that herpes is a specific infectious disease, usually contracted in early childhood, and taking the form of a febrile aphthous stomatitis. If the infection is not contracted in the earliest years, a non-immunological resistance develops, and the individual remains permanently uninfected. Those who have been infected in infancy remain permanent carriers of the virus, whose presence is shown (a) by the recurrence of herpes vesicles under the appropriate physiological stimulus, and (b) by the persistence of a high antibody titre through life.

B. Virus Infection of Monkeys.—In many respects B. virus seems to have the same relation to *Macacus rhesus* monkeys as herpes virus has to man. Experiments on the B. antibody content of monkey sera have been made both here and by Professor E. Weston Hurst at Adelaide. A considerable number contained antibody, the proportion in certain batches of monkeys being much higher than in others. All monkeys with B. antibody showed herpes antibody of about equivalent activity. Monkeys without antibody were infected with B. by intracerebral or intranasal inoculation; no definite symptoms were produced by either route, but all the inoculated animals developed both B. and herpes antibodies, the latter appearing to be more active than those against B. Herpes virus was non-infective. In the paper recording these results it is suggested that the viruses herpes, B. and pseudorabies represent divergent branches from a single stock which have developed a specially adapted, almost symbiotic relationship with their respective hosts—man, monkey, and pig.

The Pathology of Herpes Infections in Mice.—Some herpes strains, such as that (H.F.) with which most of the previously mentioned work was done, are highly pathogenic for mice. Others, such as the egg passage strain (Tr.), rarely killed, even with the largest possible doses. A study has been made of the behaviour of these two strains in the mouse, with special reference to the nature of the immunity which may be developed after infection. Titrations of virus and antibody were made on the chorioallantois. It may be noted that egg membrane methods are the only practicable ones which allow an experimental analysis of the spread of relatively avirulent virus in animal tissues.

The virulent H.F. strain introduced intracerebrally showed an initial disappearance within an hour, but from 24 hours onward there was a steady increase till death at three to four days. Peripheral inoculation (intraperitoneal and intranasal) frequently caused infection of the central nervous system, and fairly complete evidence of the route from the periphery was obtained. Following intraperitoneal inoculation, the virus reaches the spinal cord, probably through autonomic fibres from the viscera, and passes up the cord to the brain. There is no evidence that it is distributed by the blood stream. The most striking feature of virus spread after intranasal inoculation is the complete failure of infection through the olfactory nerves. There seem to be several other paths available, as evidence was obtained of infection through the fifth nerve, and of cord infection, possibly from the lung. All the evidence indicated that if virus reached the central nervous system the infection was fatal. The fact that a substantial immunity to intracerebral inoculation developed in the fairly large proportion of mice which survived such peripheral injections suggested that in mice at least the current view that herpes immunity is wholly a post-infection cellular immunity was not correct. Conclusive evidence that antibody played a significant part was obtained from experiments in which mice were effectively protected by intraperitoneal injection of formolized dead virus, or by passive administration of immune serum.

The avirulent strain, Tr., kills only when in high concentration, and then usually from five to ten days after intracerebral inoculation. The titre of virus in the brain shows a fairly regular course, reaching a peak on the fourth day, and disappearing by the seventh or eighth day. As other workers have noted, death may occur from this strain late after inoculation, yet no virus be detectable by sub-inoculation of brain emulsions. Mice surviving an intracerebral inoculation of the avirulent strain were solidly immune against the virulent strain. This immunity was detectable two days after the immunizing inoculation, so that other factors than antibody must play some part in this instance.

Influenza Virus.

In the course of work referred to in the last annual report on the pathology of infections in mice with egg-adapted influenza virus strains, considerable irregularities in the pathogenicity of the normally almost avirulent Melbourne egg strain were observed. In some experiments more than half the mice died with typical consolidation, usually from the seventh to the tenth day after infection. The cause of these fluctuations in virulence was not determined, but it was of particular interest to find that the course of virus increase and decrease in the lung, as determined by egg membrane titrations, was practically the same, irrespective of whether or not lesions had developed. In several instances mice dying about the ninth day (when the virus normally disappears) had no demonstrable virus in their lungs.

In these experiments particular attention was paid to the changes in the upper mediastinal lymph node. In infections with the virulent W.S. egg strain relatively large amounts of virus are found in the node from the second day until death. The much less severe Melbourne egg strain infections rarely show any virus in the lymph node. There is, however, a regular enlargement to a considerable extent, and antibody can be detected in extracts from the fifth day onward. The evidence suggests that possibly a large proportion of the antibody produced in the course of the infection is derived from this node.

Work has continued on the serological differentiation of influenza virus strains. Mice immunized by intranasal instillation of the avirulent Melbourne egg strain are solidly resistant to the homologous mouse strain, but much less so to the strain W.S. The specificity of active immunity was less evident when immunization was by intraperitoneal inoculation. In the course of this work it was shown that mice could be safely immunized with a virulent strain if this was dropped on to the nostrils of *unanaesthetized* mice. Recent work at Hampstead on the English strains obtained in the 1936-37 epidemic indicates that there are more serological types of human influenza virus than the two differentiated here, but a survey of the published data seems still to indicate that the 1936-37 epidemics in England and America were due to virus whose modal form was of the New World type, with many variations in antigenic detail. The 1933-36 European epidemics were of the relatively distinct Old World type.

Q. Fever.

Miss Freeman has continued work with the rickettsia of this disease, which has recently been named *R. burneti* by Derrick. The conditions under which the largest yields of rickettsiae can be obtained from mouse spleens have been studied. The most important requirement is that the mice should be inoculated with a large dose and killed about seven days later. Smaller doses require a longer time to give a maximal yield. Mouse spleens removed after the optimal period may show rickettsiae in a smear, but prove non-inffective on sub-inoculation, probably because of the co-existence of antibody in the tissue. Miss Freeman was able to show that immune sera from man, bandicoot, and mouse are capable of preventing infection of mice if mixed with a lightly centrifuged suspension of infective spleen.

Field work on the disease in Queensland has been actively and successfully carried out by Derrick and his co-workers, our collaboration being confined to testing numerous human and animal sera for Q. agglutinins. The agglutination test has, however, been of great value in these investigations in incriminating the bandicoot (*Isoodon macrurus*) as the natural reservoir of the infection. A heavily infected bandicoot population has been found at Moreton Island (Cowan Cowan), 18 out of 43 sera giving positive agglutination. We have followed with great interest the progress of the Queensland investigations on the susceptibility of the bandicoot to experimental infection and the discovery of a tick capable of transmitting the infection from animal to animal.

Psittacosis.

It is of interest that during 1938 fatal epizootics of psittacosis amongst parrots living in the wild state were observed in Victoria, Tasmania, and South Australia. Birds found recently dead were in each instance proved by full laboratory examination to have died from psittacosis. The parrots from Victoria and Tasmania were examined here, while the information about the South Australian outbreak was received from Professor Weston Hurst. Although previous work has shown how widely Australian parrots are infected with latent psittacosis, this is the first occasion on which fatal epizootics amongst wild birds have been proved due to the virus.

Myxomatosis.

Miss Lush has made a study of the serological relationship between the viruses of myxomatosis and of Shope's fibroma. It is well known that recovery from infection with the fibroma virus leaves a partial immunity to myxomatosis. Previous work had, however, indicated that no virus-neutralizing bodies were produced, but only a gross test for antibody had been used. Miss Lush finds that fibroma immune serum is capable of inactivating myxoma virus, giving a recognizable reduction of titre on both chorioallantois and rabbit skin, and that fixation of complement with myxoma egg antigen can be demonstrated with both fibroma and myxoma immune sera. In the course of this work it was shown that a soluble antigen suitable for complement fixation could be obtained by filtration of egg membrane extracts through Seitz filters. Such filtrates were non-infective.

Immunological Studies.

When opportunities have been available, a certain amount of work has been done by all members of the Department on the nature of the difference in the antibody response of rabbits, on the one hand to bacterial toxoids, and on the other to various particulate antigens, including viruses, bacteriophages, and rickettsiae. The two chief differences are, first, that toxoid shows a very sharp distinction between the responses to primary and secondary antigenic stimuli, while the particulate antigens give a primary response which appears early, and is quantitatively almost as large as the secondary one. The second difference on which Miss Freeman has been particularly engaged is the fact that peripheral inoculation of particulate antigen results in antibody production by the lymph node draining the region, while toxoid inoculated either as primary or secondary stimulus does not induce any significant production of antitoxin in the lymph node. It is possible that the two phenomena are directly related, and work is in progress to try to elucidate their significance for general immunological theory.

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"The Role of the Bandicoot in the Epidemiology of Q. Fever: A Preliminary Study," "Medical Journal of Australia," 28th January, 1939, 150.

DORA LUSH:

"The Serological Relationship of Myxoma and Shope's Fibroma Viruses," "Australian Journal of Experimental Biology and Medical Science," 1939, 17, 85.

Puerperal Sepsis.

A small outbreak of puerperal sepsis at a maternity hospital was investigated by Dr. Lucy Bryce and Dr. Phyllis Tewsley. Swabs were taken from the noses and throats of all the professional contacts of the cases. Of these contacts, 32 in number, 15 (47%) were found to harbour haemolytic streptococci. Specific serum for the more complete identification of these strains was not then available, but it seemed probable that the carrier rate for pathogenic strains would prove to be abnormally high. The hospital was closed, and when it was reopened measures designed to prevent the transference of organisms from the noses and throats of professional attendants to midwifery patients were adopted.

In the meanwhile serum was prepared, and a study was made of the strains from patients, from the contacts, and sixteen other strains from various streptococcal diseases, to ascertain what proportion of the streptococci belonged to group A Lancefield, which is responsible for the majority of severe human streptococcal infections. At the suggestion of Dr. F. M. Burnet, sensitivity to bacteriophage, kindly sent from America by Miss Alice Evans, was also tested.

As a result of this study, it was found that seven of the fifteen strains isolated from the 32 contacts belonged to Group A, i.e., the carrier rate was 22%, a much higher figure than that usually found (about 7%) among normal individuals. In view of this finding, the professional staff was re-examined five and nine months later, when there was no infection among the patients. On the second occasion, among 67 individuals, twelve strains of haemolytic streptococci were found, of which two belonged to Group A. That is, 3% of the staff were at this time carriers of Group A streptococci. In the third series of 74 persons, eighteen harboured haemolytic streptococci, among which were five Group A strains (an incidence of 6.7%).

A study was also made of the relationship between the presence of haemolytic streptococci and the clinical condition of the upper respiratory tract in the individuals in the three series described above, and in eighteen others connected with cases of puerperal sepsis which were investigated in other hospitals. It was concluded that in persons with healthy respiratory tracts, the presence of haemolytic streptococci was in most cases closely associated with recent contact with streptococcal illness, and that in such persons the organisms usually tended to disappear fairly rapidly on cessation of such contact. On the other hand, in persons in whom clinical examination revealed some abnormality in the nose or throat, there was a greater tendency for haemolytic streptococci to be present irrespective of such contact. It was suggested that the preliminary medical examination of prospective midwifery workers and the exclusion of those with detectable abnormality would lessen the risk of infection of patients, and would cause much less disorganization of training and hospital routine than their withdrawal during a period of midwifery work.

LUCY M. BRYCE and PHYLLIS TEWSLEY:

> "A Bacteriological and Clinical Investigation of the Professional Personnel of Maternity Hospitals, with Special Reference to Carriers of Haemolytic Streptococci," "Medical Journal of Australia," 9th April, 1938, p. 639.

DEPARTMENT OF PHYSIOLOGY.

Dr. W. Feldberg left us in July, 1938, to go to the Physiological Congress at Zurich where he communicated some of our results. The work on tissue injury has been continued by Dr. Trethewie and myself.

The Liberation of Histamine and the Formation of Lysocithin and of a Muscle Stimulant Substance by Snake Venom.

Further studies on the action of cobra venom by Feldberg, Holden, and myself led to the conclusion that, in addition to histamine, there were liberated from the injured cells of perfused tissues two other active substances. One of these was lysocithin, whose presence could be demonstrated in perfusates and extracts of tissues by haemolysis of sheep red blood corpuscles and the other a muscle stimulant principle which caused contraction and slow relaxation of the isolated jejunum of the guinea-pig and characteristic transitory after-changes in the excitability of the muscle. When egg yolk was treated with cobra venom *in vitro* a substance with these properties was formed together with lysocithin, and Holden has shown that crude trypsin has a similar action upon egg yolk.

We have not investigated the pharmacology of this muscle-stimulating principle, which we have provisionally called "slow-relaxing substance"—S.R.S., since a sufficiently pure preparation has not been prepared as yet, and we do not even know whether there are one or more substances with this activity.

Feldberg and I were able to study some of the actions of lysocithin, which Holden was able to prepare in a fairly pure state. It causes transitory decrease in the excitability of the guinea-pig's jejenum, but a strong contraction with short latency in the isolated virgin uterus of the guinea pig. It produces haemorrhagic oedema of the lung when injected intravenously into the intact guinea-pig. For the rest, its behaviour resembles that of venoms. Histamine, protein, and pigments are liberated from the perfused liver of the dog when it is injected intraportally. Intravenous injection in the cat or dog causes a steep fall of systemic pressure which in the former animal is attended by a rise of pressure in the pulmonary artery and haemorrhagic oedema of the lung, and in the latter by a rise of pressure in the portal vein. Injected into the femoral artery of the dog, it causes transient vasodilatation.

There is great variation in the haemolytic and neurotoxic actions of venoms, and it seemed desirable to ascertain whether the tissue injury caused by them could be related to the haemolytic action of the venoms and whether their neurotoxic action might be a manifestation of cell injurious action of the same kind. With this in view, Dr. Trethewie has compared two Australian venoms—those of the black snake (*Pseudechis porphyriacus*) and death adder (*Acanthophis antarcticus*). Both caused the liberation of histamine and of coagulable protein from perfused tissues and the formation of lysocithin and S.R.S., the venom of the black snake being much more potent, as accords with its more powerful haemolytic action. These two venoms exhibited a striking parallelism in haemolytic power and in their ability to liberate histamine and protein from perfused organs. It appears likely, therefore, that both these manifestations of tissue injury by snake venoms are mediated by the formation of lysocithin. Since black snake venom is only feebly neurotoxic and death adder venom is powerfully so, it is improbable that the neurotoxic action of these Australian snake venoms is caused by the constituents responsible for haemolysis and injury of the tissues in general.

Tissue Injury by Staphylococcal Toxin.

Feldberg and I continued the study of this problem. The injection of the toxin into the pulmonary artery of the perfused lung of the dog causes the liberation into the perfusate of protein, histamine, and a slow-relaxing substance. When injected intraportally into the perfused liver, cellular debris, pigment, and the slow-relaxing substance appear in the perfusate. The cellular debris is rich in histamine, which is brought into solution by boiling or by treatment with lysocithin or cobra venom. The toxin does not form either lysocithin or slow-relaxing substance when it is incubated with egg yolk *in vitro,* and it seems likely that S.R.S. is liberated and not formed by the action of the toxin on perfused tissues. The toxin has a stimulating action on the isolated jejunum of the guinea-pig, followed by decreased excitability of the muscle to histamine. The gut is desensitised by repeated administration of toxin.

Tissue Injury by Mercuric Chloride.

The intraportal injection of this heavy metal salt into the perfused liver of the dog causes coagulation of the cells and liberation of histamine and of coagulable protein into the perfusate. No slow-relaxing substance is liberated in this form of injury. The amount of heavy metal in the perfusate appears to depend on the amount of coagulable protein present in it.

Tissue Injury by Radiant Energy.

It has long been known that ultra-violet radiations cause injury to the tissues. Trethewie, Holden, and I, in our experiments upon the isolated perfused lung of the dog, showed that ultra-violet, visible and infra-red radiations caused the liberation of histamine into the perfusate. The amounts of histamine so set free were small in comparison with those liberated by agents which act uniformly throughout the perfused organs. Only a thin layer of the surface cells is directly exposed to the injurious action of the radiations, but we were able to show a small but significant loss of histamine from the surface layer of the lung. The injury so caused resembles the coagulation necrosis by mercuric chloride, in that no slow-relaxing substance appears in the perfusate.

Photodynamic Action.

The severe circulatory symptoms which are caused by irradiation of the skins of animals sensitised to light by porphyrins and other photodynamic agents may be easily explained if pharmacologically active substances are liberated in the cell injury so caused. Trethewie and I took up this problem and showed that histamine is liberated by photodynamic action. In our experiments on the direct effects of radiant energy we had used very slow rates of flow in our perfusion experiments, since only small amounts of histamine were liberated. In testing the photodynamic effects of haematoporphyrin, using these slow rates of flow, we obtained at first negative results. Histamine is, however, rapidly destroyed by photodynamic action, and when we increased the rate of flow so as to give but little time for destruction of the histamine in the outflowing perfusate, we found that it was present in significantly greater amounts than by the action of light alone. The destruction of histamine by photodynamic action *in vitro* is readily prevented by the presence of serum proteins, and it therefore seems unlikely that histamine liberated *in vivo* would be destroyed by photodynamic action, since it would be protected by the proteins of the cells and tissue fluids. No slow-relaxing substance was found in the perfusate in these experiments, and since our crude S.R.S. was not destroyed by photodynamic action *in vitro,* we think it improbable that any slow-relaxing substance is liberated in this form of injury. We also studied the action of light in the presence of haematoporphyrin upon the isolated jejunum of the guinea-pig. Supniewski had shown in 1927 that some forms of isolated plain muscle sensitised with haematoporphyrin contract and then finally lose their excitability and rhythmicity when exposed to light. We tested this effect on the isolated jejunum of the guinea-pig to ascertain whether histamine or slow-relaxing substance were likely to be liberated from these tissue cells. We observed only loss of excitability, which was generally irreversible. No contraction occurred which might indicate the liberation of pharmacologically active substances from these tissue cells by photodynamic action.

Tissue Injury by Anaesthetics.

Dr. Trethewie and I have also studied the effect of volatile and gaseous anaesthetics on the isolated perfused lung of the dog. High concentrations of chloroform cause extreme tissue injury, with the liberation of histamine, protein, and S.R.S., but concentrations of the order which are used in anaesthesia are without effect. Ether in very high concentrations also causes severe tissue injury, with the liberation of histamine and S.R.S., but the concentrations of the order which are used in anaesthesia also cause some degree of tissue injury, some S.R.S., but no histamine being set free. Ethylchloride in high concentrations causes tissue injury with liberation of S.R.S. and histamine, but it is doubtful whether any serious injury is possible during the short periods for which it is used in anaesthesia. Nitrous oxide and ethylene, when administered for prolonged periods, caused no significant tissue injury, although after further prolonged perfusion traces of histamine may appear in the perfusate.

Tissue Injury in Anaphylaxis.

We continued our study of this type of tissue injury in the guinea-pig. From the isolated perfused lungs of sensitised guinea-pigs we were able to show that in addition to histamine, which was shown to be set free by Bartosch, Feldberg, and Nagel, a slow-relaxing substance appeared in the perfusate. Intraportal injection of antigen into the perfused sensitised liver caused no increase of substances with this property in the perfusate. At first we thought that the slow-relaxing substance must be formed in anaphylaxis, because simple extracts of sensitised lung after injection of the antigen contained much more S.R.S. than was present in control extracts, and no such difference could be observed in liver perfusion experiments. Furthermore, extracts of sensitised lung, incubated with antigen *in vitro,* also contained more S.R.S. than control extracts. We were, however, uncertain whether by simple extraction we were getting out all the S.R.S. present, and when we repeated these *in vitro* experiments, using a method which enabled all the active material to be extracted, we were no longer able to detect these differences. It was clear, therefore, that the slow-relaxing substance was liberated from the tissues, and not formed, and that our regular results with simple extraction with the Tyrode solution must depend on the greater ease of extraction of the substance after anaphylactic injury. An attempt was made to analyse the factors involved in the anaphylactic contraction of plain muscle, by using a variety of agents which cause reversible or irreversible depression of excitability, in the hope that a differential desensitization to histamine or to S.R.S. might be achieved. The agents used were lysocithin, slow-relaxing substance prepared from egg yolk with cobra venom, carbon dioxide, histaminase, and photodynamic action. The evidence obtained was not decisive, but it seemed likely that the liberation of S.R.S. played no significant part in the anaphylactic contraction of the virgin uterus of the guinea-pig. The contraction of the sensitised gut closely resembles its response to S.R.S. or to snake venoms, and the liberation of S.R.S. in the tissue probably accounts for the slow-relaxation and the after changes in excitability of the muscle which are part of the anaphylactic response.

Synthesis of Acetylcholine.

Last year Mann, Tennenbaum, and Quastel brought forward evidence pointing to the synthesis of acetylcholine by brain tissue, especially in the presence of glucose. The possibility that part of the increase in acetylcholine observed by these workers may have been accounted for by preformed acetylcholine in incompletely ground fragments of tissue was not wholly excluded. Dr. Trethewie, at Dr. Feldberg's suggestion, repeated these experiments, using the brain tissue of the guinea-pig, samples of the eserinised ground material incubated with and without glucose being boiled before testing. An increase of 40% acetylcholine was shown after two hours' incubation with 0.02 M. glucose.

Other Work in Progress.

Dr. Trethewie and I are studying the effects of the toxins of the anaerobes of the gas gangrene group in causing tissue injury. We have already shown that some of these toxins cause the liberation of histamine from the perfused organs of susceptible species, though not from those of animals which are naturally resistant.

W. Feldberg, H. F. Holden, and C. H. Kellaway:

''The Formation of Lysocithin and of a Muscle Stimulating Substance by Snake Venoms,'' ''Journal of Physiology,'' 1938, 94, 232.

W. Feldberg and C. H. Kellaway:

''The Liberation of Histamine by Staphylococcal Toxin and Mercuric Chloride,'' ''Australian Journal of Experimental Biology and Medical Science,'' 1938, 16, 249.

C. H. Kellaway, H. F. Holden, and E. R. Trethewie:

''Tissue Injury by Radiant Energy and the Liberation of Histamine,'' ''Australian Journal of Experimental Biology and Medical Science,'' 1938, 16, 331.

C. H. Kellaway and E. R. Trethewie:

"Photodynamic Action and the Liberation of Histamine," "Australian Journal of Experimental Biology and Medical Science," 1939, 17, 61.

"Tissue Injury by Volatile and Gaseous Anaesthetics," "Australian Journal of Experimental Biology and Medical Science" (in the press).

E. R. Trethewie:

" 'Free' and 'Bound' Histamine and Acetylcholine," "Proceedings of the Physiological Society," 15th October, 1938, "Journal of Physiology," 94.

"Synthesis of Acetylcholine by Brain Tissue," "Australian Journal of Experimental Biology and Medical Science," 1938, 16, 343.

"Comparison of Haemolysis and Liberation of Histamine by Two Australian Snake Venoms," "Australian Journal of Experimental Biology and Medical Science" (in the press).

C. H. Kellaway:

"The Chemistry of Animal Poisons," "Annual Review of Biochemistry," 1939.

"The Symptomatology and Treatment of the Bites of Australian Snakes," "Medical Journal of Australia," 8th October, 1938, p. 585.

BIOCHEMICAL DEPARTMENT.

Bile Pigment Iron Compounds.

Mr. H. F. Holden has collaborated with Dr. R. Lemberg, of the Kolling Institute of Research, at the Royal North Shore Hospital, Sydney, in work on the ultra-violet absorption spectra of some bile pigment-iron compounds and other products of the break down of haemoglobin. Much of Dr. Lemberg's share of this work was done in his own laboratory, but the National Health and Medical Research Council made it possible for him to visit the Hall Institute, and he was able to prepare some of the less stable compounds here. Holden and Lemberg found that the only compounds which show the Soret band in the near ultra-violet are those to whose structure an intact porphyrin ring is assigned on the basis of independent chemical evidence. Fission of this ring, whether any atom is, or is not, eliminated, abolishes the Soret band in the ultra-violet spectrum.

Blood Pigments.

In 1938 Mr. Holden deferred a good deal of his spectrophotometric work pending the arrival of the new echelon cell ultra-violet spectrophotometer. For the work with Dr. Lemberg and for some studies with Mr. Rawlinson on the degree of denaturation of oxyhaemoglobin, methaemoglobin and globin by metallic ions he was able to use an older type of instrument which was kindly lent us by Professor W. A. Osborne, from the Department of Physiology at the University. The new spectrophotometer, which was provided by the National Health and Medical Research Council, arrived in November last. It has proved most useful for securing rapid photographs of the ultra-violet absorption spectra of substances while they were undergoing changes which it was desired to record without interruption. Work has begun on the methaemoglobin-acid haematin transformation, but had to be abandoned during the hot weather, and has now been resumed. A number of metalloporphyrins have been prepared in order to study their combination with globin and other proteins. The ultra-violet absorption spectrum of the haemocyanin of the Australian crayfish is also being studied. Perhaps the most interesting results to date are that the band at 3400A. is associated with the presence of the oxygen molecule in this protein, and that carbon monoxide, if it combines with the protein, yields a compound with the same spectrum as that of the "reduced" haemocyanin. It appears to be extremely likely that carbon monoxide does not combine with this haemocyanin, but the question requires thorough investigation by other methods.

Compounds of Metallic Ions with Proteins.

Mr. W. A. Rawlinson, working under a grant from the National Health and Medical Research Council, has continued his work on the compounds of metallic ions with proteins. Some of his results may be briefly summarised. The formation of precipitates occurs within definite limits of hydrogen ion concentration, which vary according to the ion and the protein. The precipitates formed with zinc sulphate and oxyhaemoglobin or globin can be redissolved without either protein being denatured, while if copper sulphate be used the former protein, but not the latter, is irreversibly denatured. Further investigations showed that the denaturation is due to oxidation, and appears to be allied to the irreversible denaturation of oxyhaemoglobin by other agents, for example, acids or organic solvents, which was shown by Mr. Holden to be due to an oxidation of the protein coupled to that of the iron atom in the prosthetic group. Mr. Rawlinson carried out titrations of the reactions between oxyhaemoglobin and haemoglobin with cupric ions both spectroscopically and electrometrically. For the work on haemoglobin a special apparatus was devised and constructed in the laboratory to secure the rigid exclusion of oxygen during the experiment. An apparatus was also constructed for supplying cheaply the large volumes of hydrogen free from oxygen which were required. The results of several determinations agreed well, and showed that at pH 4.7, when oxygen is carefully excluded and denaturation avoided by careful manipulation, one cupric ion oxidizes one ferrous atom in the haemoglobin. In less acid solutions an excess of cupric ions is required for quantitative oxidation. In the presence of oxygen this excess leads to denaturation.

It is proposed to utilize the magnetic balance for the determination of the magnetic susceptibilities of metal protein compounds with a view to the further investigation of their constitution. A grant was made available by the National Health and Medical Research Council, and a suitable balance is now in the course of construction. Meanwhile Mr. Rawlinson is subjecting the haemocyanin of the Australian crayfish to a thorough investigation from many aspects. This protein, which acts as an oxygen carrier, contains copper in its prosthetic group, and is an interesting example of a naturally occurring metallo-protein. It crystallizes beautifully, and in neutral solution appears to be very stable. It is insoluble at its iso-electric point, pH 4.7, and is rapidly denatured at pH 4.0.

Determination of Traces of Carbon Monoxide in Blood.

Mr. D. E. Bland commenced work in February on this problem under a grant from the National Health and Medical Research Council. A good deal of preliminary experimental work has been done. It is hoped to use the well-known reaction between the gas and iodine pentoxide, and by an adaptation of a recent catalytic determination of the exceedingly minute traces of iodine which are obtained to determine the carbon monoxide in specimens of blood of a reasonable volume. The difficulty of the task may be indicated by the statement that the amount of iodine to be dealt with will not exceed a few millionths of a gram. While methods for separating and determining small quantities of carbon monoxide are available, they tend at present to be slow, complex, and costly. The development of a simple and accurate method would be of great value in connection with many problems in industrial hygiene.

Slow-Relaxing Substance.

Mr. Holden, and for a short time, Mr. Setter, have worked on the preparation and purification of this substance. It is formed when snake venom or trypsin (B.D.H.) is incubated with egg yolk but not with egg albumin. Its formation by trypsin is not accompanied by change in pH like that caused by snake venom. Attempts at purification of the crude extracts by a variety of methods have resulted in almost complete disappearance of pharmacological activity. The substance is not identical with lysocithin, and appears not to have any close chemical relationship with cephalin or lysocephalin. Further work is in progress.

New Apparatus.

During 1938 some trouble was experienced with apparatus owing to the increased number of workers and the fact that much of the apparatus had been in use for some years. This necessitated much time being spent on overhauls and minor repairs, and has also caused some delays owing to absence of sufficient equipment. The National Health and Medical Research Council recognised this difficulty, and has provided a substantial grant, which is being expended on necessary equipment.

Other Work.

A great many minor pieces of work have been carried out by Mr. Holden and his colleagues of the biochemical department, which, while they do not contribute to the output of research from the department, form an effective contribution to the work of other departments in the Institute.

Work on the apparatus and equipment of the Institute has included cleaning and overhauling a mercury vapour lamp, repairing a centrifuge starter, repairing potentiometers, cleaning and overhaul of balances and other delicate apparatus. In addition, Mr. Holden assisted in setting up a glass electrode outfit for a worker in another institution.

More general work has included purifying picric acid and making an unusual stain, concentrating solutions, preparing special proteins, drying and weighing venoms, preparing pure organic solvents and high vacuum dessication of anti-sera.

H. F. Holden and R. Lemberg:

"The Ultra-violet Absorption Spectra of Bile Pigment-iron Compounds and of Some Bile Pigments." "Australian Journal of Experimental Biology and Medical Science" (in the press).

W. A. Rawlinson:

"The Effect of the Ions of Copper, Zinc, and Mercury on the Blood Pigment." "Australian Journal of Experimental Biology and Medical Science," 1938, 16, 303.

"Further Studies on the Reactions of Copper Ions with Haemoglobin, Oxyhaemoglobin and Methaemoglobin." "Australian Journal of Experimental Biology and Medical Science," 1939, 17, 53.

CLINICAL RESEARCH.

Severe Haemorrhage from the Stomach and Duodenum.

Dr. Ian Wood, the Marion Carty Research Fellow, and Miss Bick have been continuing their work on the biochemical changes which occur in massive haemorrhage and in the state of paralytic ileus. This work has been made possible by a grant from the National Health and Medical Research Council for Miss Bick's salary and for the purchase of apparatus.

The chemical changes which take place in the blood and urine as a result of severe haemorrhage from the stomach and duodenum are being studied, and the effects of treatment with massive blood transfusion, with Meulengracht's high caloric feeding and with the intravenous infusion of physiological fluids. In some instances this has necessitated more than 100 microchemical analyses on specimens from the same patient. One patient suffering from repeated haemorrhages from a gastrojejunal ulcer, and who probably had a pre-existing renal lesion, was transfused with eleven pints of blood and ten and a half pints of glucose saline. At one stage of his illness the blood urea rose to 240 mgms. per 100 c.c. He eventually recovered.

Paralytic Ileus.

Dr. Wood and Miss Bick have continued their work on this form of intestinal obstruction, and have studied in particular the efficiency of methods aimed at restoring the extensive loss of water, salts, and food which occurs in this condition from the stomach and upper portions of the alimentary canal. To compensate for this loss, large volumes of fluid are infused into the veins. The present work aims at restoring the deficiency of chloride, protein, and glucose, and at eliminating the acidosis which occurs.

Peripheral Circulatory Failure.

Dr. Wood has been making a clinical study of the use of neosynephrin (Stern) in patients suffering from peripheral circulatory failure. Neosynephrin is a vaso-constrictor drug closely allied to adrenalin and ephedrin. Unlike adrenalin, it has a wide margin of safety when injected intravenously. A single intravenous injection causes only a transitory rise in blood pressure, usually lasting from ten to thirty minutes. However, when the drug is infused slowly into the veins by the continuous-drip method, a prolonged effect is obtained which may last for several hours. Now that continuous intravenous infusion has become a simple and practicable measure, this method of administration of drugs may prove to be of great value, especially when absorption from the alimentary canal is impaired by circulatory failure.

The Storage of Blood for Emergency Blood Transfusion.

Miss Eleanor Williams and Dr. Ian Wood have evolved a practical technique for the collection, storage and administration of blood for emergency blood transfusion. In the early stages of this work they received valuable advice from workers overseas, notably from Dr. R. W. B. Ellis, of London, who worked with the blood transfusion unit of Dr. Duran Jorda, in Spain, and from Dr. Fantus, of the Cook County Hospital, in America, who founded the first "blood bank." Mr. Holden has also advised concerning chemical aspects of this problem, and Miss Bick has continued and extended her work, which has been concerned with chemical analyses of the stored blood. Her results show that during storage changes occur in the composition of some of the nitrogenous elements of the red cells. When these changes have progressed to a certain well-defined degree, haemolysis follows. It has been found possible to delay the onset of haemolysis by the addition of small amounts of glucose, and, what has proved to be even more important, by the introduction of rigorous cleaning of apparatus and of the utmost purification of the water and of the reagents used.

The blood has usually been mixed with a glucose-citrate solution, and Miss Williams has studied the effects of varying the concentration and pH of this solution. Variation in the keeping properties of blood from different individuals has been controlled by collecting the blood from each donor in two separate reservoirs, each containing a different concentration of the glucose-citrate solution. All the blood for this work has been provided by members of the Red Cross Voluntary Donor Service, to whom our thanks are due for their help and keen interest. During the past four months 29 donors have each given a pint of their blood for storage purposes. The blood has been kept at a temperature of 2° C. for periods varying from three to twenty-one days, and then administered to patients who were in urgent need of blood transfusion. In less than thirty minutes blood can now be collected from cool storage, warmed, conveyed to the ward, and administration to the patient begun. By this means more than one life has already been saved. When this work was commenced, many technical difficulties were encountered, but during the past two months the results have been most encouraging. During this period thirteen patients have been transfused with a total of sixteen pints of stored blood, and on only two occasions have there been mild pyrexial reactions. It is hoped that with improved technique these reactions may be entirely eliminated. The average duration of storage has been ten days, and the maximum period seventeen days.

With the development of the scheme, the space available for cool storage at the Institute has been greatly taxed, and I appealed to the Victorian Branch of the Red Cross Society for assistance. The Society immediately arranged for the purchase, at a cost of approximately £150, of an electric refrigerator specially constructed for the purpose of blood storage. This is now installed at the Institute. I wish to express our gratitude to the Red Cross Society for this timely help. Our thanks are also due to Mr. Walter Bassett, who advised as to the most suitable refrigeration for this purpose.

During the year Dr. Wood read a paper on the bleeding peptic ulcer before the Royal Australasian College of Surgeons.

IAN J. WOOD:

> "Some Observations on the Treatment of the Bleeding Peptic Ulcer." "Australian and New Zealand Journal of Surgery" (in the press).
>
> "Some Observations on the Massive Intravenous Infusion of Physiological Fluids." "Medical Journal of Australia" (in the press).

Vitamin C. in Urine.

Dr. Drevermann, working under a part-time grant from the Marion Carty Fund, has studied the preservative effect of acids on vitamin C in urine as a preliminary to estimating the vitamin content of the urine in patients with peptic ulcer.

Glacial acetic acid 1:10 was found to be unsatisfactory. There was considerable loss of ascorbic acid even in two hours, and in 24 hours the loss was from 40 to 60%. Sulphuric acid 50% (1:10) preserved the ascorbic acid, but caused too much charring of the pigments of the urine. In 10% strength the preservative action of the acid was incomplete, but in a strength of 20% almost the whole of the ascorbic acid in freshly voided urine was still present at the end of 24 hours, and there was but little charring. Twenty-four hour specimens were then preserved with this concentration of acid. The ascorbic acid contents, as estimated from these, were compared with the results obtained from estimating the content of the fresh urines. The amounts of ascorbic acid in the preserved specimens were within 8% of those estimated in fresh urines. In all the estimations made on urines acidified with sulphuric acid, an allowance had to be made for the slight decolourisation of the dye by this acid. The 24 hour excretions of ten normal subjects showed a variation between 19.7 and 28.9 mgms. per diem. The normal excretion is usually stated to be between 30 and 33 mgms. per diem.

Blood Changes in Thyrotoxicosis.

Dr. P. S. Woodruff, working at the Institute under a Randal and Louisa Alcock Scholarship of the University of Melbourne, is investigating changes in the numbers of red and white blood corpuscles, blood platelets, and in the amount of haemoglobin, in cases of thyrotoxicosis before and after operation. Histological study of the excised thyroid glands from these cases is also being made.

Neurological Research.

Dr. E. Graeme Robertson has examined the olfactory bulbs from eleven fatal cases of human poliomyelitis. Inflammatory changes were found in only one olfactory bulb, and there was no indication that this reaction was certainly due to poliomyelitis. It was therefore assumed that if the virus gained access to the central nervous system by this route, it was able to pass through the olfactory bulb without producing inflammatory changes.

In conjunction with work being carried out by Dr. Burnet and Dr. Jackson, the brains of monkeys infected with poliomyelitis virus by various routes have been examined. Intraocular injection in rhesus monkeys frequently produced ptosis, and sometimes paralysis of upward movement of the eyes before other signs appeared. The inflammatory reaction was longest established and most intense in the region of the third cranial nerve nuclei, but there were no inflammatory changes along the optic nerve pathways. These changes appeared later at other levels of the brain stem in the grey matter of the spinal cord, and in the motor cortex. The histological picture of the inflammation produced by inoculation in other situations has also been studied. In cynomolgus monkeys the inflammatory reaction was more cellular, intense, and initially more widely spread through the nervous system. Experiments have also been made on the distribution of staining by trypan blue after injection into monkeys at various stages of paralysis. The results have been correlated with histological studies, and have re-emphasised the attack of the virus on motor regions.

Throughout the year Dr. Robertson has continued his clinical and pathological studies on the neurological material available in the hospital. Much time has been devoted to the preparation of cinema films for teaching.

ROUTINE HOSPITAL SERVICES.

Bacteriology and Clinical Pathology.

During the year 18,479 diagnostic investigations for 11,378 patients have been carried out. These include 2922 Wassermann tests, 287 gonococcal complement fixation tests, and 2465 tests for allergy. Bacteriological and haematological tests comprised the remainder. The ever-increasing demand for diagnostic tests allows little time for special investigations.

During the past year the typing of all pneumococci isolated from respiratory diseases into Cooper's 32 types has been continued. The predominant type has been Type III, this being isolated more than twice as frequently as any other individual type. Other types frequently met with were II, I, VIII, VII, VI, and X.

A series of Addis tests were done to aid in the assessment of the residual damage to the kidneys in former cases of nephritis.

From what appears to be the first authentic case of Weil's disease in Victoria, *Leptospira icterohaemorrhagica,* was isolated. This was a fatal case occurring in a worker at the fish market. An endeavour is being made to isolate the spirochaete from rats supplied by the City Council through the courtesy of the City Health Officer (Dr. John Dale). No positive result has been obtained, but so far the series is small.

The technique for obtaining biopsy material for bone marrow examination has been studied, together with the most suitable methods of preparing the material for examination. The opinion was formed that the bone marrow obtained by trephining the sternum gave more satisfactory results than that yielded by puncture. It seems necessary to examine smears for cellular details and sections for architectural changes to gain the maximum amount of information.

An unusual case of urticaria provoked by heat, exercise, and exertion has been under observation and desensitisation by injections of histamine was attempted, and gave temporary benefit. A course of injections of ''Doryl'' (Carbaminoylcholine chloride) is now being given, with promising results.

Dr. J. D. Hicks succeeded Dr. T. E. Wilson as Assistant Clinical Pathologist in January.

Miss G. E. Morton, M.Sc., is at present a voluntary worker in the department, and is assisting in the preparation of protein extracts for allergic tests.

Biochemistry and Basal Metabolism.

During the first portion of this year the work of the Biochemical Department was performed by Miss Verney South, B.Sc. In October Miss Splatt returned from England, and took over the department. Many of the biochemical methods have been changed following Miss Splatt's visit to the Courtauld Institute, Middlesex Hospital, including those used for analysis of blood phosphate, phosphatase, uric acid, and blood protein and non-protein nitrogen. This work, unfortunately, calls for better temperature regulation of water baths, etc., than we have available, and it is hoped that more modern equipment of this nature will be obtained during the current year. Miss South has been working half time since October on the preparation of solutions and thorough testing of the various new methods against those previously used.

In general the majority of the specimens have been of the same nature as in previous years, some thousands of tests for blood sugar and blood urea being performed, and numerous fractional test meals and urea concentration tests. There is a steady increase in the number of specimens for examination, and this year over 500 B.M.R.'s have been determined with the Benedict-Roth calorimeter.

Dr. Drevermann is working on ascorbic acid exertion in this laboratory.

Miss Jakobowitz, biochemist to Dr. Colin Ross, is carrying out here some investigations of the intravenous blood sugar curve on patients with metabolic diseases in the Royal Melbourne Hospital.

ELECTROCARDIOGRAPHY.

During the year 913 electrocardiograms have been taken with the ''Matthews'' Portable Instrument presented by the ladies of the Box Hill and Mont Albert Auxiliary in 1932.

Dr. L. E. Rothstadt has kindly undertaken to report on these records, which, until now, have been returned to the wards without any attached comment.

MORBID ANATOMY.

During the year 467 autopsies were performed. Dr. C. H. Mollison and Dr. Wright-Smith were responsible for the examination of 1510 biopsy sections from 1194 specimens. Sections taken from autopsy material numbered 703. Routine examination of the vermiform appendix removed at operation was carried out on 385 occasions. The routine photography has been done by Miss Helen Wischusen.

DR. R. J. WRIGHT-SMITH:

''Case of Fatal Gas Poisoning in Welding in Closed Tank.'' ''Journal of Industrial Hygiene and Toxicology,'' vol. 21, No. 1, January, 1939.

''Pathology of Osteitis Deformans.'' ''Melbourne Hospital Clinical Reports'' (in the press).

Museum.

Many new specimens were added to the museum, including regional ileitis, cysts of the lung, suppurative nephritis, renal cystine calculus, tuberculosis of the uterus, and duct carcinoma of the nipple.

Teaching.

Dr. Wright-Smith has given the usual course of tutorial lectures in Pathology to fourth-year students in December and February. Lecture demonstrations in Morbid Anatomy have been given twice weekly throughout the year to fifth and sixth year students, and lectures in Elementary Bacteriology have been given to the student nurses in the hospital. Dr. H. Gardner has been responsible for the tuition in Clinical Pathology to fifth-year students.

The Library.

Our thanks for the gifts of journals and books are due to the following:—L'Académie Royale de Médecine de Belgique; The Commonwealth Department of Health; The Council of Scientific and Industrial Research; Miss Danks; Mr. Dobell, F.R.S.; Dr. Keith Fairley; The Government Institute for Infectious Diseases of the Tokyo Imperial University; The London Hospital; The Medical Research Council; The Middlesex Hospital Medical School; New York State Department of Health, Division of Laboratories and Research; Dr. R. J. Wright-Smith; La Société Royale des Sciences Médicales; The South African Institute for Medical Research; The University of Harvard, Department of Tropical Medicine; The University of Pennsylvania, Department of Pathology; The University of Tomsk.

CHARLES H. KELLAWAY,
Director.

The Walter and Eliza Hall Institute of Research in Pathology and Medicine

FINANCIAL STATEMENT FOR THE YEAR ENDED 30th JUNE, 1939.

RECEIPTS.

To Balance Brought Forward from 30th June, 1938				£3,692	14	5
,, Trustees, W. & E. Hall Trust	£3,200	0	0			
,, University of Melbourne	750	0	0			
,, Mrs. A. M. Nicholas	250	0	0			
,, Felton Bequests Committee	370	0	0			
,, Estate Alfred Edments	100	0	0			
,, Estate Anthony Mackie	85	0	0			
,, Royal Melbourne Hospital—Refund	301	18	10			
,, Fees Received and Proceeds of Materials Sold	291	15	3			
,, Interest	85	6	11			
,, State Accident Insurance Office—Refund	2	4	5			
				5,436	5	5
				£9,128	19	10

EXPENDITURE.

By Salaries and Wages	£4,261	12	6			
,, Materials	278	17	5			
,, Apparatus	5	8	8			
,, Sundries	199	2	2			
,, Publications	111	12	2			
,, Fittings and Equipment	13	12	9			
,, Repairs to Buildings	27	10	11			
,, Repairs to Apparatus	30	15	5			
,, Library Account	4	13	2			
,, Bio-Chemical Dept.	465	15	4			
				£5,399	0	6
,, Balance—						
Investments	1,875	0	0			
Cash — Agent-General, London	52	9	8			
Secretary's Advance A/c	100	0	0			
Bank of N.S.W.	1,702	9	8			
				3,729	19	4
				£9,128	19	10

THE ELSIE MARION CARTY ACCOUNT.

To Balance Brought Forward from 30th June, 1938	£405 15 7		By Salaries	£204 8 9	
,, Interest on Investments	167 12 0		,, Materials	14 3 7	£218 12 4
		£573 7 7	,, Balance		354 15 3
		£573 7 7			£573 7 7

BIO-CHEMICAL DEPARTMENT ACCOUNT.

To Interest from Endowment Fund	£366 11 7	By Salaries and Wages	£832 6 11
,, Transfer from Income Account	465 15 4		
	£832 6 11		£832 6 11

LIBRARY ACCOUNT.

To Balance Brought Forward from 30th June, 1938	£8 4 8	By Books, Journals, and Bookbinding ..	£98 19 4
,, Interest on Investments	86 1 6		
,, Transfer from Income Account	4 13 2		
	£98 19 4		£98 19 4

VIRUS ACCOUNT.

To Commonwealth Government	£1,000	0	0	By Debit Balance Brought Forward from 30th June, 1938	£451	8	2
,, Rockefeller Foundation	1,000	0	0	,, Salaries and Wages	1,542	13	0
,, Mr. E. Alec Cato	1,000	0	0	,, Balance, Bank of N.S.W.	1,006	19	10
,, Sundries	1	1	0				
	£3,001	1	0		£3,001	1	0

FUND FOR NEUROLOGICAL RESEARCH.

To Balance Brought Forward from 30th June, 1938	£264	12	1	By Salaries and Wages	£210	6	4
				,, Materials	10	6	7
				,, Balance, Bank of N.S.W.	43	19	2
	£264	12	1		£264	12	1

ENDOWMENT FUNDS.

To Balance — Bio-Chemical Endowment Fund	£8,591	10	3	By Investments	£14,844	8	10
,, Balance—Elsie Marion Carty Fund	4,233	15	3	,, Bank of N.S.W.	10	16	8
,, Balance—Library Fund	2,030	0	0				
	£14,855	5	6		£14,855	5	6

MEDICAL RESEARCH COUNCIL GRANTS.

	£	s.	d.		£	s.	d.
To Balance Brought Forward from 30th June, 1938	£728	2	3	By Salaries and Wages	£1,707	0	0
,, Commonwealth Government	3,693	3	4	,, Apparatus, Materials, etc.	1,483	7	3
,, Sundries	0	3	0	,, *Balance, Bank of N.S.W.	1,231	1	4
	£4,421	8	7		£4,421	8	7

*The balance includes the sum of £1078/13/4 paid in advance for quarter ending 30/9/39.

POLIOMYELITIS RESEARCH GRANTS.

	£	s.	d.		£	s.	d.
To Balance Brought Forward from 30th June, 1938	£307	8	4	By Salaries and Wages	£821	0	2
,, Commonwealth Government	1,893	19	2	,, Buildings	37	18	6
				,, Materials	1,021	18	8
				,, *Balance	320	10	2
	£2,201	7	6		£2,201	7	6

*The balance includes the sum of £283 paid in advance for quarter ending 30/9/39.

AUDITOR'S CERTIFICATE.

I have to report that I have completed the Audit of the Books and Accounts of the Institute for the period ended 30th June, 1939. I have verified all receipts and have had vouchers produced for all disbursements. All information and explanations required have been given. The Statement is a correct statement of Receipts and Expenditure as revealed by the Books of the Institute.

(Sgd.) W. M. JARVIE, F.C.A. (Aust.), Auditor.